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Anti-Predator Behavior and Discriminative Abilities: Playback Experiments with Free-Ranging Equatorial Saki Monkeys (Pithecia aequatorialis) in the Peruvian Amazon

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To the Dean of the Graduate School:

We are submitting a thesis written by Candace R. Stenzel entitled "Anti-predator behavior and discriminative abilities: playback experiments with free-ranging equatorial saki monkeys (*Pithecia aequatorialis*) in the Peruvian Amazon".

We recommend acceptance in partial fulfillment of the requirements for the degree of Master of Science in Biology.

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ANTI-PREDATOR BEHAVIOR AND DISCRIMINATIVE ABILITIES: PLAYBACK EXPERIMENTS WITH FREE-RANGING EQUATORIAL SAKI MONKEYS (*PITHECIA AEQUATORIALIS*) IN THE PERUVIAN AMAZON

A Thesis

Presented to the Faculty

Of the

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In Partial Fulfillment

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Of

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By

Candace R. Stenzel

ABSTRACT

Anti-predator behaviors of free-ranging groups of equatorial saki monkeys (*Pithecia aequatorialis*) were recorded in the Área de Conservación Regional Comunal Tamshiyacu Tahuayo in the Peruvian Amazon to determine whether individuals responded in predator-specific ways to calls of aerial and terrestrial predators. Previous studies have shown that several species of Old World monkeys possess these discriminative abilities, but few have explored this question in New World monkeys. The ability to recognize predators and to respond appropriately is important for survival. Thus, we predicted that equatorial saki monkeys should respond in predator-specific ways, both vocally and behaviorally, to aerial and terrestrial predators. To investigate this, we simulated the presence of predators by playing recordings of harpy eagle calls (*Harpia harpyja*; an aerial predator) and ocelot growls (*Leopardus pardalis*; a terrestrial predator) to wild saki groups. Response variables measured included individuals' vocalizations, movement, and gaze orientation. Between June and August 2016, we conducted 24 playback trials on 16 individuals. Chi-squared goodness of fit and Fisher's exact tests revealed that alerted or predator-specific responses were given more often than no response at all following the playbacks of predator calls. Thus, while additional confirmation is needed, our data provide preliminary evidence that saki monkeys possess predator specification capabilities.

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BACKGROUND

Pithecia Background

Saki monkeys (*Pithecia*) are a forest-dwelling group of New World primates found exclusively in South America (Marsh 2014). Their north to south range extends from the Guiana Shield to northern Bolivia, and east to west from Altamira on the Rio Xingu to the base of the Andes Mountains in Ecuador and Peru (Marsh 2014). Together with the titi monkeys (*Callicebus*), uakaris (*Cacajao*), and bearded saki monkeys (*Chiropotes*), they make up the Pitheciidae family (Norconk 2007). All pitheciids are arboreal, diurnal, small- to medium-sized monkeys (Norconk 2007). Sakis are intermediate in size between the titis (smallest) and bearded sakis and uakaris (largest) (Norconk 2007), and range in body weight (1347-3000 g), head and body length (30-70.5 cm), and tail length (25.5-54.5 cm) (Hershkovitz 1987).

Taxonomy

Saki monkeys are very understudied due to their inconspicuous behavior, cryptic appearance, difficulty of habituation to human observers, and occupation of a dense canopy environment (Peres 1993, Norconk 2006, Pinto et al. 2013). Thus much is still to be learned regarding their biology, ecology, and behavior (Peres 1993, Norconk 2006). Furthermore, there has always been much confusion in *Pithecia* taxonomy (Marsh 2014).

Historically, Hershkovitz (1987) identified five *Pithecia* species separated into two groups: 1) the Guianan region *Pithecia pithecia* group with two subspecies of *P. pithecia*, and 2) the Amazonian region *Pithecia monachus* group with two subspecies of *P. monachus*, two subspecies of *P. irrorata*, *P. aequatorialis*, and *P. albicans.* Even prior to that, *P. aequatorialis* was identified as *P. monachus*, and *P. monachus* was identified

as *P. hirsuta* (Hershkovitz 1979, 1987). Marsh (2014) revised the taxonomy of the *Pithecia* genus and identified 16 *Pithecia* species: five original species names remain - *P. irrorata*, *P. albicans*, *P. aequatorialis*, *P. monachus*, *P. pithecia*, although the species descriptions and geographic distributions for *P. aequatorialis* and *P. monachus* have been redefined; three species were moved up from subspecific ranks - *P. vanzolinii*, *P. napensis*, *P. milleri*; three species were reinstituted - *P. chrysocephala*, *P. hirsuta*, *P. inusta*; and five new species were identified - *P. cazuzai*, *P. isabela*, *P. mittermeieri*, *P. rylandsi*, *P. pissinattii.*

Social Structure

While little research has been done regarding *Pithecia* social structure, most species were previously thought to organize themselves into monogamous pairs with their dependent offspring (Happel 1982, Norconk 2006). In more recent years however, several studies have reported groups containing multiple breeding age adults of one or both sexes (e.g., Norconk 2006, 2007, Di Fiore et al. 2007, Aquino et al. 2009, Thompson 2013, Porter et al. 2015, Lehtonen 2016, Van Belle et al. 2016). These multi-adult groups might develop as a result of the increased benefits associated with larger groups (e.g., resource and territory defense), reflect a transition period of temporary associations with solitary individuals, or be a result of postponed dispersal amongst offspring (Di Fore et al. 2007, Thompson et al. 2012, Ferrari et al. 2013, Porter et al. 2015, Thompson 2016, Van Belle et al. 2016). Group size varies between species, but typically ranges from one to seven individuals (Happel 1982, Bennett et al. 2001, Heymann et al. 2002).

Reproduction and Development

Norconk (2006) found both male and female white-faced sakis (*P. pithecia*) dispersing from natal groups at three-and-a-half to four years of age, reproduction of single birth offspring beginning at four to six years of age, and interbirth intervals (IBI) ranging from 12-36 months (average IBI = 21.2 months). Mothers are the primary care providers for their newborns, although older male and female siblings have been observed assisting with infant carrying as well (Brush and Norconk 1999, Buzzell and Brush 2000). While paternal care is relatively absent (Norconk 2006, 2007, Thompson 2011, 2016), there have been reports of increased paternal interactions with older infants (e.g., play, food sharing) (Norconk 2007, Fernandez-Duque et al. 2013). Newborns typically become completely self-locomoting at five months (i.e., follow behind group members and no longer rely on being carried), though short bouts of infant locomotor independence (i.e., time spent off and away from mother) can start as early as five to eight weeks (Brush and Norconk 1999, Norconk 2006).

Home Range Size

Home range size and daily movements for groups are still not fully understood, but research on white-faced sakis and buffy sakis (*P. albicans*) have shown that ranges vary between 100 and 250 ha, with daily movements of 1000-2000 m (Peres 1993, Vié et al. 2001, Ferrari et al. 2013). Smaller ranges of less than 100 ha have been reported for white-faced sakis as well (Oliveira et al. 1985, Vié et al. 2001, Ferrari et al. 2013).

Diet

Saki monkeys have large procumbent lower incisors and laterally splayed canines, a specialized dental morphology believed to allow them to crack open and pierce into

tough fruits to acquire seeds (Ferrari et al. 2013, Kay et al. 2013). While most animals typically swallow seeds whole or discard them while only eating the edible portions of the fruit, sakis masticate seeds prior to swallowing (Kay et al. 2013). The first two feeding strategies effectively allow for future opportunities of seed dispersal and germination, while the third essentially destroys the seeds and prevents any future opportunities for seed germination (Kay et al. 2013). Saki monkeys are thus often referred to as "seed predators" due to their destructive feeding strategy (Kay et al. 2013). While fruits and seeds make up the bulk of their diet, research on buffy sakis and white-faced sakis have shown them to eat a wide variety of other foods as well, including leaves, flowers, and insects (Oliveira et al. 1985, Peres 1993, Norconk 2007).

Pithecia aequatorialis

Equatorial sakis are one of the most understudied primates of the Amazonian tropics (Aquino et al. 2009). Under the IUCN Red List, they are classified as being of 'least concern' (Marsh and Veiga 2008). Their exact geographic distribution is still not completely certain, though Aquino et al. (2009) suggests their range to be sympatric with monk sakis (*P. monachus*), and is bounded by the Curaray and Napo Rivers in the north, the Marañón River in the south, the Amazonas River in the east, and the Corrientes and Tigre Rivers in the west **(Figure 1)**. Chism et al. (in review) further extended their distribution southeast of the Marañón, Amazonas, and Ucayali Rivers, into the Área de Conservación Regional Comunal Tamshiyacu-Tahuayo.

Although Marsh (2014) redefined *P. aequatorialis* as *P. monachus*, and *P. monachus* as *P. inusta*, Kieran (2012) found that the calls of my focal-species overlapped extensively with those recorded for *P. aequatorialis* in Ecuador, and did not overlap with

calls of *P. monachus* (*P. inusta* per Marsh (2014)). Therefore for this study, I decided to adhere to the taxonomic, geographic, and morphological descriptions of *P. aequatorialis* as described by Hershkovitz (1987) and Aquino et al. (2009), and identify my focal species as *P. aequatorialis*.

Equatorial sakis are a dichromatic species (Hershkovitz 1987). Distinguishing features on males include long blackish hairs that are banded buffy on the back and limbs; buffy to orange hairs on the chest, throat, beard, sides of head, forehead, and crown; paired buffy crests from the forehead to crown separated by bare central and lateral bands; bare cheeks and chin; a thin malar stripe, and buffy wrists, ankles, and metapodials (Hershkovitz 1987). Distinguishing features on females are similar to males, though their forehead and crown are covered with long blackish hairs banded pale buff; long blackish hairs on cheeks banded buffy; a well-developed malar stripe; orange hairs on the throat and chest; and mixed orange and blackish hairs on the stomach and inner sides of limbs (Hershkovitz 1987).

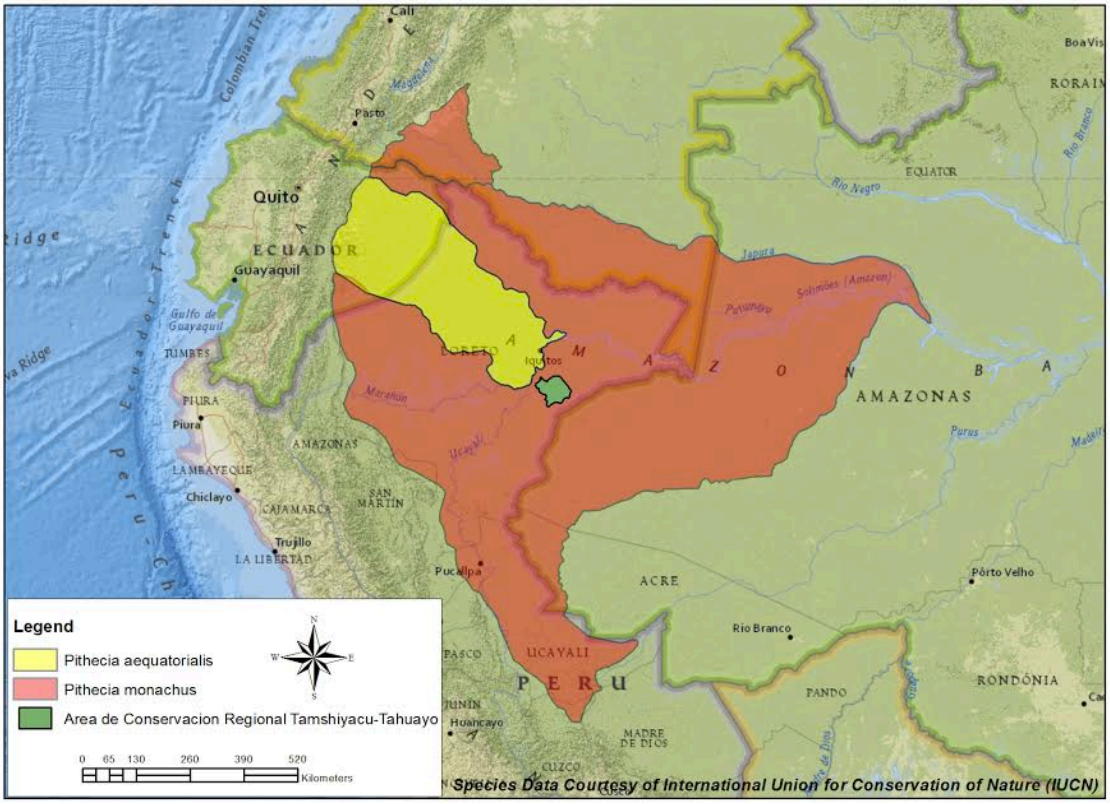

Figure 1: Map showing the geographic distribution of *P. aequatorialis* (yellow) and *P. monachus* (red) in relation to the boundaries of the Área de Conservación Regional Comunal Tamshiyacu-Tahuayo (ACRCTT) (green). The ACRCTT is the communal reserve in which our study took place. Chism et al. (in review) further extended the distribution of *P. aequatorialis* into the ACRCTT. Species distribution data used with permission of the International Union for Conservation of Nature (IUCN).

Área de Conservación Regional Comunal Tamshiyacu-Tahuayo

The research for this study was carried out within the Área de Conservación Regional Comunal Tamshiyacu-Tahuayo (ACRCTT) in Loreto, Peru **(Figure 2)**. Originally named the 'Reserve Comunal Tamshiyacu Tahuayo' (RCTT), it was established in 1991 as a communal reserve (IUCN Category VI for protected areas) through joint efforts between local Peruvian communities and conservationists, and gives local communities exclusive access to resources within its protected boundaries while preventing overexploitation from outside commercial companies (Newing and Bodmer

2003, Newing 2009). From 1991 to 2007, the RCTT spanned across 322,500 ha of lowland rainforest, though in 2007 the reserve was reclassified as a state park for the District of Loreto, expanded to 420,080 ha, and renamed the 'Área de Conservación Regional Comunal Tamshiyacu-Tahuayo' (ACRCTT) (Newing and Bodmer 2003, Penn 2009).

The habitat of the ACRCTT primarily consists of un-flooded terra firme forest, but also includes várzea forest (seasonally flooded by white-water rivers) and igapó forest (seasonally flooded by black-water rivers) (Prance 1979, Puertas and Bodmer 1993). The seasonal flooding typically starts in March and lasts through May (Kvist and Nebel 2001).

Figure 2: Map showing the geographic location of the ACRCTT within Peru. Map downloaded from Google (https://maps.google.com).

Animal Behavioral Research

The roots of behavioral studies first appeared in the works of many late 19th century naturalists such as Charles Darwin, Charles O. Whitman, and Oskar Heinroth (Fericean et al. 2015). While these naturalists may have provided the basis for a more methodological approach to studying behavior, the modern field of behavioral research is rooted in 'ethology', which was generally attributed to having started in the 1930s following the works of Nikolaas Tinbergen, Konrad Lorenz, and Karl von Frisch (Fericean et al. 2015). Within that decade, Tinbergen began examining animal behavioral responses to stimuli (e.g., Tinbergen 1951), von Frisch began examining the meaning behind the dances of bees (e.g., von Frisch 1954), and Lorenz began research on instinct and imprinting (e.g., Lorenz 1981) (Hakansson and Westander 2013).

Behavioral ecology is one area of research that stemmed from the field of ethology and first became established as an independent field of study in the late 1960s-early 1970s (Birkhead and Monaghan 2010). Behavioral ecologists look at patterns of variation in behavior and are concerned with the functional, adaptive, and evolutionary aspects of these variations within different ecological environments (Birkhead and Monaghan 2010, Shettleworth 2010). Research in this field is driven largely by theory, with models often being used to formulate and test hypotheses pertaining to those behaviors an animal should use in different situations for maximizing fitness levels (Birkhead and Monaghan 2010). For example, a researcher might look at the fitness consequences of one particular behavior and then consider the costs and benefits gained from selecting that behavior over another (Birkhead and Monaghan 2010).

Predator Recognition in Prey Species

Many of the major topics explored in behavioral ecology focus on adaptive responses and behavioral plasticity, both of which are important attributes for animals to have when dealing with the wide array of environmental conditions encountered daily (Ghalambor et al. 2010). One area of particular interest amongst behavioral ecologists is the adaptive and flexible responses of anti-predator behaviors (Ghalambor et al. 2010). Predator avoidance is important for any animal. The failure to successfully avoid a predator consequently results in death and ultimately prevents an individual from providing any additional contributions to fitness (e.g., production of more offspring) (Ydenberg and Dill 1986, Blumstein et al. 2008). Thus from an evolutionary perspective, there are strong selective pressures for individuals that are successful in avoiding predators (Ydenberg and Dill 1986).

Predator avoidance capabilities also have important evolutionary and conservation implications (Berger 1998, Berger et al. 2001, Jones et al. 2004). Over the last century with widespread decline in the numbers and ranges of larger predator populations, many prey species have lost contact with former predators, or encounter them less frequently (Berger 1998, Berger et al. 2001). While fewer predators may seem beneficial for prey, it could also cause loss of predator recognition capabilities, and thus result in a more vulnerable prey population if a particular predator were to be eventually encountered or reintroduced in a region (Berger 1998, Berger et al. 2001, Jones et al. 2004, Blumstein 2008). If predator recognition is a learning process, adaptive responses should be able to redevelop quickly (Griffin et al. 2000, 2001). Thus, the ability to develop effective predator recognition capabilities and flexible anti-predator behaviors could become

increasingly vital for the survival of several prey species as predator-prey overlaps continue to transform over the next several centuries (Blumstein 2008).

Although predators typically remain silent while actively hunting, they almost all have distinctive vocalizations that numerous prey species have demonstrated abilities to recognize and subsequently respond in an appropriate anti-predator manner. Three possible mechanisms have been suggested for how prey might appropriately respond to predators: 1) individuals may be predisposed to these recognition and avoidance capabilities at birth (Schel and Zuberbühler 2009, Hettena et al. 2014), 2) predator recognition and avoidance may be learned, either through observations of others, or through direct encounters with the predators themselves (Griffin et al. 2000, 2001, Berger et al. 2001, Schel and Zuberbühler 2009), or 3) individuals may possess these recognition and avoidance capabilities at birth, but require a direct experience first before effectively putting them to use (Blumstein et al. 2000, Schel and Zuberbühler 2009).

Playback Experiments

Playback experiments are a common tool used to test a species' anti-predator capabilities. These experiments involve playing recordings of sound stimuli (e.g., predator calls) to target individuals and/or groups, and recording behavioral and vocal responses (Fischer et al. 2013). Playback experiments are used in all areas of behavioral research, and enable researchers to directly test hypotheses related to cognitive understanding, behavior, and vocal meaning (Fischer et al. 2013). The responses that individuals and/or groups give to these playbacks can reveal what they understand (e.g., playback of a predator call - can individuals recognize predator calls and respond appropriately?), how they act (e.g., playback of a neighboring conspecific's vocalizations

- how territorial does an individual act when hearing a conspecific intruder in his or her territory?), or give insight to what a particular vocalization might mean (e.g., playback of a group member's alarm call - how do other group members respond when hearing an individual's alarm call?).

Several predator playback studies have documented species of all taxa to possess anti-predator capabilities. For example, Lohrey et al. (2009) found that wolf spiders (*Schizocosa ocreata*) cease movement and courtship behaviors upon hearing the calls of a blue jay (*Cyanocitta cristata*), an avian predator; Cantwell (2010) reported brown anoles (*Anolis sagrei*) responding with increased vigilance to calls of several predatory birds; Jones et al. (2004) documented eastern quolls (*Dasyurus viverrinus*) to cease movement and/or respond with increased alertness to calls of the masked owl (*Tyto novaehollandiae castanops*), an avian predator, and Tasmanian devil (*Sarcophilus laniarius*), a terrestrial predator; and Thuppil and Coss (2013) found Asian elephants (*Elephas maximus*) could discriminate between tigers (*Panthera tigris*), a species threatening to calves, and leopards (*Panthera pardus*), a species not threatening to calves, by silently moving away from tiger growls, but aggressively vocalizing towards leopard growls. These predator playback studies have ultimately revealed the cognitive capabilities that prey species have with recognizing predator calls and knowing when and how to respond appropriately to avoid predation.

Primate Predator Playback Experiments

Playback experiments are especially common in primate behavioral and acoustic studies (Fischer et al. 2013). The first set of primate playback experiments were initiated in 1890 by Richard Garner while using Thomas Edison's phonograph to record and play

back the vocalizations of capuchin and rhesus monkeys housed in zoos (Fischer et al. 2013). While Garner is considered to be the "pioneer" of primate playback experimentation, it was Seyfarth and Cheney's (1980a) predator playback experiments on vervet monkeys (*Chlorocebus pygerythrus*) beginning in the 1970s that profoundly established anti-predator behavioral and acoustic research on free-ranging primates (Fischer et al. 2013). They found that vervet monkeys were capable of recognizing and responding in predator-specific ways to both their aerial and terrestrial predators, and to their own acoustically different alarm calls given to those same predators. For example, a leopard's presence and the vervet monkey alarm calls given to leopards both caused conspecifics to look down towards the ground and/or move higher up in a tree, while an eagle's presence and the vervet monkey alarm calls given to eagles both caused conspecifics to look up towards the sky and/or move into denser tree cover.

Alarm calls are a common anti-predator strategy in several avian and mammalian species, and are often used to warn conspecifics of an approaching predator, or to inform the predator that it has been detected (Caro 2005, Cäsar and Zuberbühler 2012). One area of particular interest amongst many primatologists has been the possibility that primate alarm calls have semantic features (i.e., the calls themselves contain referential meaning towards external stimuli) (Koda and Sugiura 2010). Zuberbühler et al. (1997) assert that for calls to have a predator referential function, each call must have structural acoustic differences that correspond with specific predators or predator classes, and conspecifics must react to the different predator calls as they would if the predator were actually present. Besides Seyfarth and Cheney's series of playback experiments with vervet monkeys (e.g., 1980a,b, 1990, Cheney and Seyfarth 1988), several other playback studies

have suggested primate alarm calls may operate on a referential basis (e.g. Zuberbühler et al. 1997, Zuberbühler 2000, Fitchtel and Kappeler 2002, Kirchhof and Hammerschmidt 2006, Schel et al. 2010, Wheeler 2010, Cäsar et al. 2012).

Objective of Study

In this study I used playback recordings of predator calls to try to determine whether free-ranging equatorial saki monkeys respond in predator-specific, adaptive ways to calls of their aerial and terrestrial predators. Previous studies have shown that several species of Old World monkeys possess these discriminative and referential abilities, but few have explored this question in New World monkeys (e.g., Kirchhof and Hammerschmidt 2006, Wheeler 2010, Cäsar et al. 2012). The ability to recognize predators and respond appropriately has important survival implications.

Furthermore, because New World monkeys inhibit dense tropical forests where opportunities for visual communication are often limited, vocal communication and predator specification should have important roles in predator contexts (Cäsar et al. 2012). This pertains to saki monkeys as well, who occupy a dense canopy environment and often remain cryptic and out of visual contact with other group members while foraging (Peres 1993, Norconk 2006, and Pinto et al. 2013). Thus, I predicted that equatorial saki monkeys should respond in predator-specific ways, both vocally and behaviorally, to recorded calls of their aerial and terrestrial predators.

For this study, I used recordings of ocelot growls (*Leopardus pardalis*) as the terrestrial predator, and harpy eagles calls (*Harpia harpyja*) as the aerial predator. When choosing the predator calls, I referred to previous pitheciid predation research done by de Luna et al. (2010) and Cäsar et al. (2012), who found pitheciids to be susceptible to a

wide variety of terrestrial and aerial predators including boa constrictors (*Boa constrictor*), tayras (*Eira barbara*), ocelots, pumas (*Puma concolor*), ornate hawk-eagles (*Spizaëtus ornatus*), black hawk-eagles (*Spizaetus tyrannus*), harpy eagles, and crested eagles (*Morphnus guianensis*). While playback recordings of any of these predators could have been used for this study, ocelots and harpy eagles had the largest selection of available recordings, hence why these two predators were selected for this study.

MATERIALS AND METHODS

Study Site

This study was carried out at the Tahuayo River Amazon Research Center (TRARC) (S 04º23.334', W 073º 15.438') between June 22nd and August 6th 2016. The TRARC is located along the Tahuayo River, a tributary of the Amazon River, within the Área de Conservación Regional Comunal Tamshiyacu-Tahuayo (ACRCTT) in Loreto, Peru, and consists of seasonally flooded, lowland igapó forest **(Figure 3)**. The majority of the data were collected on a 2 km x 2 km trail grid system just south of the research center and Tahuayo River (**Figure 4)**. This grid encompasses 400 ha of igapó forest with five habitat subtypes (higher restinga, lower restinga, bajial, palm swamp, and streamside), and has 42 intersecting trails, each two km long and 100 m apart. Trails running parallel to the river were numbered 0-20, while trails running perpendicular were labeled A-U. There were two other unmarked trails in this area that we occasionally used as well, each about two km long. One trail, commonly referred to as "The River Trail", ran between the grid and the Tahuayo River, while the other trail ran parallel to the Tahuayo River on the opposite side of the river from the TRARC. In addition to the hiking trails, we used a small motorboat for traveling five km up- and downriver to search the igapó forest that ran immediately along either side of the Tahuayo River.

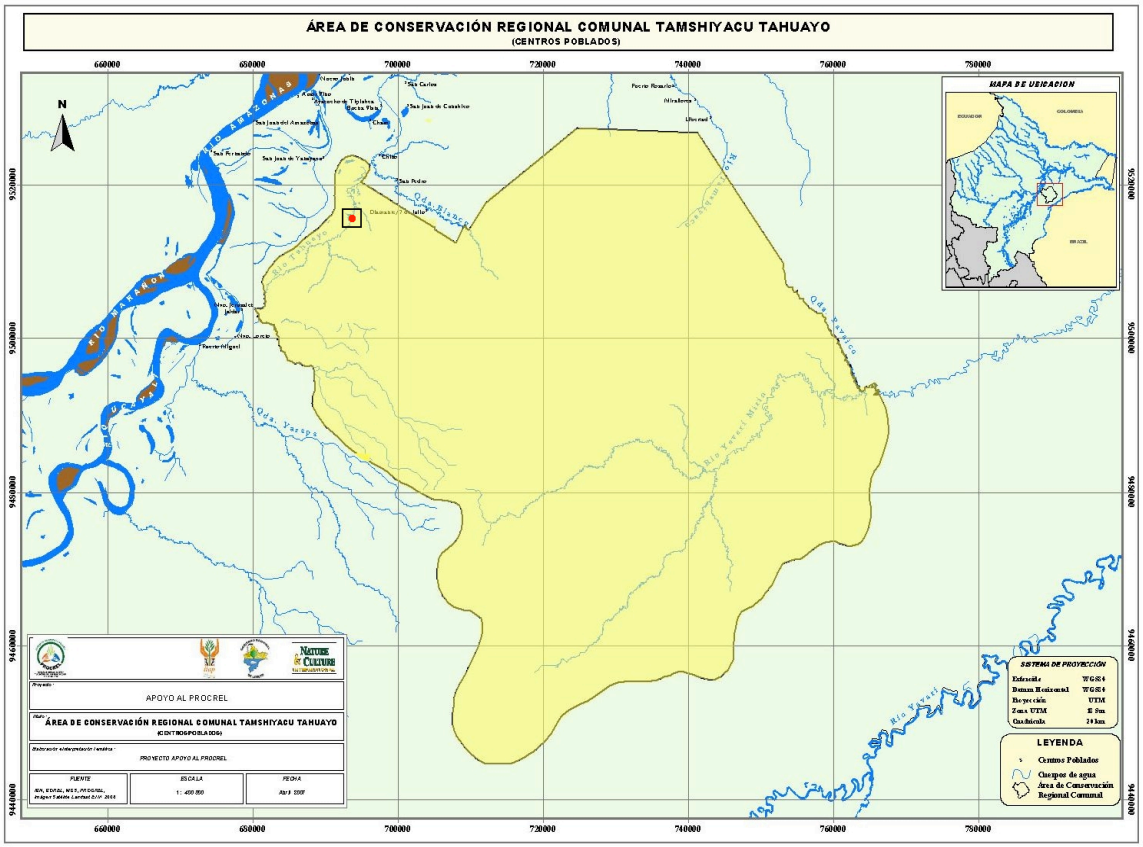

Figure 3: Map showing the boundaries and geographic location of the ACRCTT. The TRARC study site is marked with the red dot. Map downloaded from Amazonia Expeditions (www.perujungle.com).

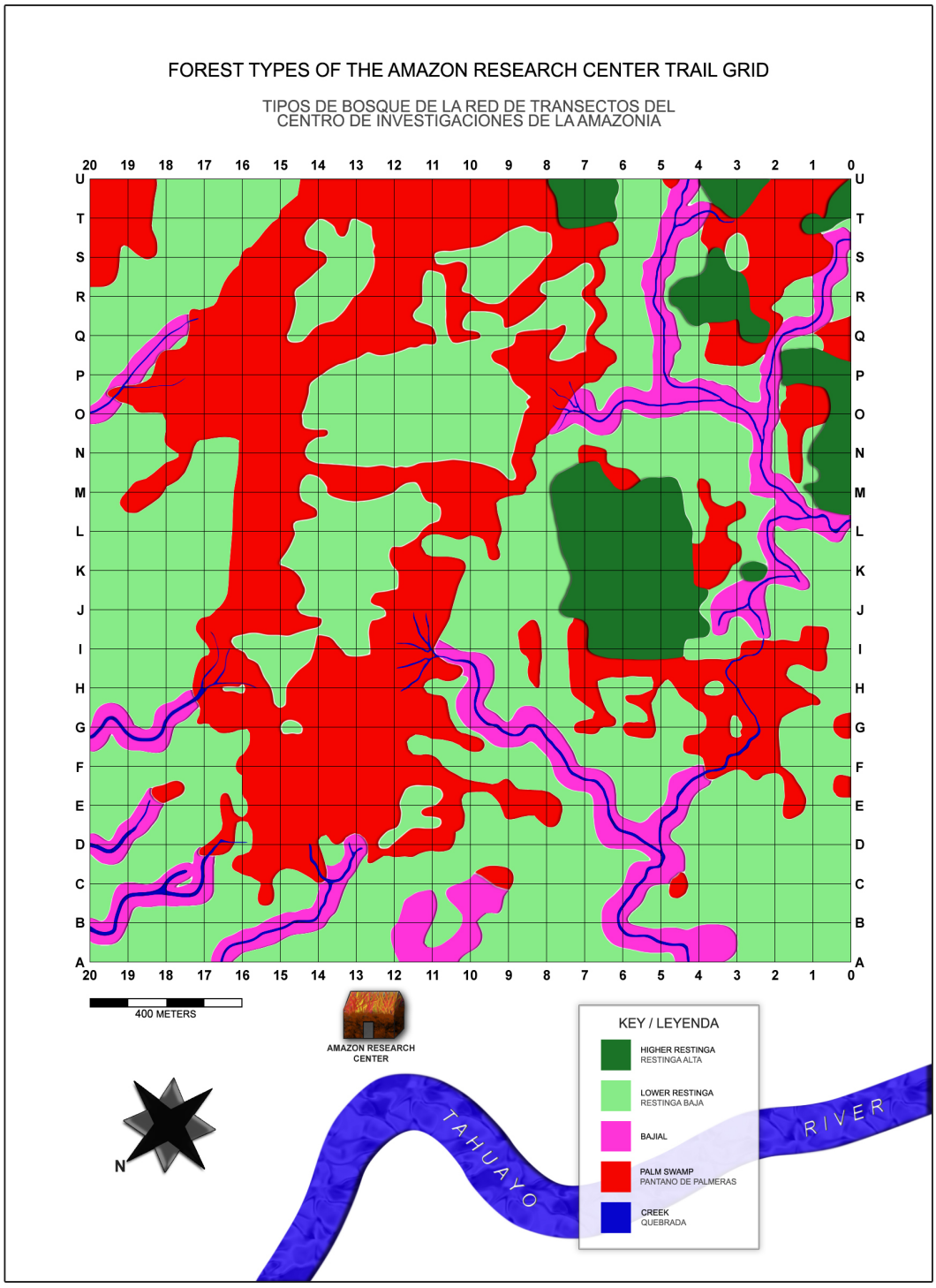

Figure 4: Map of the 2 km x 2 km trail grid system at the TRARC. The five habitat subtypes are labeled by the different colors. Map created and used with permission by Alfredo Dosantos Santillan.

Field Assistance

All data were collected with the help of Manuel Huayllahua Silva, a local guide familiar with the trail grid and surrounding landscape. Dr. Janice Chism also accompanied me at the beginning of this study from June 22nd to June 30th. During this time, she helped me get started with initial data collection and trained me in saki identification and observation. In addition, I had the help of three high school interns from the United States who were interested in the field of biology. These interns helped with the study by carrying/holding equipment, and assisting in data collection by recording video and taking photos of the sakis we encountered. The interns all arrived and departed at different times, so Manuel and I were never with more than one or two interns on any given day while collecting data in the field. Having as few individuals as possible while in the field was important in order to better detect saki groups, and not alarm them with a large group presence.

Playback Stimuli

Playback stimuli for this study included a) ocelot growls—a terrestrial predator to sakis (de Luna et al. 2010, Cäsar et al. 2012), b) harpy eagle calls—an aerial predator to sakis (de Luna et al. 2010, Cäsar et al. 2012) and c) cracks of thunder (a control recording). All recordings of harpy eagle calls were downloaded through the Cornell Lab of Ornithology: Macaulay Library; ocelot growls were taken from both the Macaulay Library and audiomicro.com, and thunder recordings were taken from soundboard websites at mediafire.com and freesound.org. All sounds downloaded through the Macaulay Library were field recordings that were previously recorded throughout the Amazonia regions of Brazil and Peru, but recording locations were not able to be

determined for the sounds taken from the soundboard websites (e.g., audiomicro.com, mediafire.com, and freesound.org).

To avoid pseudo-replication, multiple sound recordings were made for each playback stimulus: eagle calls (n=10), ocelot growls (n=5), and cracks of thunder (n=6) (Wiley 2003), and prior to going out in the field, all playback recordings were edited to a standard 15 seconds and uploaded onto a Samsung Galaxy S7 phone.

Experimental Protocol

All of the methods used for this study were adapted from previous playback studies on primate groups including: Seyfarth et al.'s (1980a) and Cheney and Seyfarth's (1982) experiments with vervet monkeys in Kenya; Zuberbühler et al.'s (1997) experiments with Diana monkeys (*Cercopithecus diana diana*) in Côte d'Ivoire; Schel et al.'s (2010) work with guereza colobus monkeys (*Colobus guereza*) in Uganda; Cäsar et al.'s (2012) experiments with black-fronted titi monkeys (*Callicebus nigrifrons*) in Minas Gerais; and Maciej et al.'s (2013) work with Guinea baboons (*Papio papio*) in Senegal.

We searched for saki groups in the morning (6:30 am-12:30 pm) and mid-afternoon (3:00 pm-5:30 pm) by boating on the river and walking the TRARC trail grid and unmarked trails. The areas searched were where saki group sightings have been previously reported, both recently as noted by Peruvian guides and tourists, and from reviewing notes on group locations from a previous study (Kieran 2012). If there was a recent sighting, we would always search that area first before searching other areas.

When walking on trails, we moved quietly at a two to three km/hr pace, stopping frequently to scan the trees and listen for saki vocalizations. When boating on the river, we scanned the trees and canopy line for groups while traveling approximately five km

up- or downriver from the TRARC. The boat motor was always used for upriver travel, while downriver travel used canoe paddles with the engine off.

Once a saki group was located, it was our intent to monitor the monkeys' behavior for at least ten minutes prior to performing any playback to ensure that they remained unbothered by our presence (following Schel et al. 2010). However, because of their often skittish behavior and propensity to flee or at least move into denser cover, this 10-minute monitoring period turned out to be unrealistic. Thus, playback trials proceeded immediately after equipment setup as long as 1) individuals did not appear agitated by our presence, 2) at least one individual remained within visual contact, and 3) no encounters with predators or other saki groups took place during equipment setup (following Zuberbühler et al. 1997).

Presentation of Playback Stimuli

We simulated the presence of a predator by playing in a randomized order a) a harpy eagle call, b) an ocelot growl, and c) a crack of thunder. Recordings for each predator type were previously sorted and then put into a randomized playback order to prevent habituation to the presented sound stimuli (Caselli et al. 2015), and at least five minutes passed in between each playback recording. Furthermore, if a group was re-tested, a different recording was used for each sound stimulus so that groups never heard the same recordings more than once (Wiley 2003).

Playback recordings were played at maximum volume using the Samsung Galaxy S7 phone's music library connected via Bluetooth to a Bose SoundLink Color Bluetooth speaker. In order to standardize the height from which the sound was being played, the speaker was attached to the top of a one-and-a-half m pole. When on the boat, we held

the pole with the speaker attached on top, but if on foot, we leaned the pole against a tree one to three m away for us. In an ideal situation, we wanted to place the speaker 15-20 m away from us so as to remain away from the source of sound. However, after the first few failed attempts at playbacks due to the difficult behavior of the monkeys, we soon learned that we had to set up the speaker quickly before the monkeys moved off.

Data Collection

Whenever possible, an intern video recorded all behavioral and vocalization data using a Sony HD Handycam PJ 760 while I observed behavior through Celestron Nature DX 8x42 binoculars. However, it was often difficult to focus the video camera because there were so many tree layers in the canopy. Furthermore, the video camera became unusable halfway through the study. Once the video camera could not be used, I observed the monkeys' behavior using only the binoculars. Field notes on behaviors were taken immediately after the playbacks and observations.

Video recording and/or monitoring through binoculars began one minute prior to playing a single recorded call, and continued for an additional one minute post-call. Over this timeframe, individual and group behaviors were recorded using the ad-libitum sampling method as defined by Altmann (1974). Once playbacks were completed, the time, location and GPS coordinates were recorded using a Garmin GPS Map 62sc. When able to be determined, the age and gender of each observed individual were recorded as well.

It was my intention to have no more than three playback trials (one trial for each sound stimulus) take place on a single day for a single group, and to have at least 48 hours pass between playback sessions carried out on the same group (following

Zuberbühler et al. 1997). Due to the limited time and the need for as many samples as possible, if the same group was found within 24 hours, I would opt to perform another playback session; however, there was only one occasion when this happened. There were two additional occasions where we found a saki and performed a single playback stimulus on that saki, only to have another saki appear a few minutes later. When this happened, I would replay that same predator call using a different recording in order to get response data from the second individual, and then take more response data from the saki that was originally present. This may have caused some bias and pseudoreplication in the data, but due to the limited time and a low success rate in finding groups to perform playbacks, I decided to include this small number of repeats in the data (3 out of 24 playbacks, 12.5%).

Upon return from the field, I measured the following variables:

1) <u>Vocalizations:</u> The number of vocalizations elicited after a recording were summed and classified according to the corresponding 11 main equatorial saki monkey call types as defined by Kieran (2012).

2) <u>Behavior:</u> The number of behaviors elicited after a playback recording was summed and classified into the following behavioral categories:

A) <u>Movement:</u> The number of individuals that moved either a) horizontally towards the speaker, b) horizontally away from the speaker, c) vertically up the tree, d) vertically down the tree, or e) in any direction into more dense cover (following Schel et al. 2010). An individual had to have moved at least two

consecutive steps in the same direction to classify a behavioral response as 'movement' (following Cheney and Seyfarth 1982).

B) <u>Gaze:</u> The number of individuals that looked either a) towards the speaker, b) up towards the sky, c) down towards the ground, or d) elsewhere, which was defined as gazing around in any other direction (following Cäsar et al. 2012).

All behaviors and vocalizations in the video recordings were analyzed for 20 seconds prior to- and one minute after the start of a single playback recording using the QuickTime Player Version 10.1 movie software on a Mac OS X 10.7.5 laptop. The time frame of analysis controlled for behavior leading up to the playback and revealed any differences between the behavior that occurred immediately before and after a recording was played (Seyfarth et al. 1980a). To correct for behavior prior to a playback recording, any call type and/or behavior that occurred within three seconds of the start of a playback recording was not counted (following Cheney and Seyfarth 1982). In situations where multiple call types and/or behaviors occurred from a single individual, each vocalization and/or behavior was counted once for the appropriate call type and/or behavioral category (following Maciej et al. 2013).

Statistical Analyses

Because multiple behaviors typically occurred from a single saki monkey following a playback, a single response was selected from each individual in order to avoid pseudoreplicated data (e.g., reporting multiple behaviors from a single individual for analyses). This was done by first examining the individuals' responsiveness to the

playback treatment. If an individual responded to a playback call with any of the behavioral responses listed in the above paragraph, we considered that individual as being responsive to the playback. If an individual did not respond in any way, we considered that individual as unresponsive.

If an individual was responsive to a playback call, we then categorized the overall response as being appropriate, inappropriate, or generalized. Responses considered appropriate were behaviors specific to a particular playback treatment (e.g., looking up towards the sky for an eagle call), while inappropriate responses were behaviors considered inconsistent with a particular playback treatment (e.g., looking down towards the ground for an eagle call). Generalized responses were behaviors considered appropriate for any playback treatment (e.g. looking around/scanning the area for any of the three playback treatments). See **Table 1** for listed behaviors for each response category within each playback treatment.

	Approp. Resp.	**Inapprop. Resp.**	**General Resp.**
Eagle	Look up	Look down	Look around
	Move down	Move up	Look at speaker
	Move into cover	Move towards speaker	Move away from speaker
Ocelot	Look down	Look up	Look around
	Move up	Move down	Look at speaker
	Move into cover	Move towards speaker	Move away from speaker
Thunder	Look up	Look down	Look around
	Move down	Move up	Look at speaker
	Move into cover	Move towards speaker	Move away from speaker

Table 1: Appropriate, inappropriate, and generalized behaviors for each playback treatment.

Because individuals typically responded to a playback with multiple behaviors, if any response within the mix of behaviors was considered inappropriate, that individual was categorized as having responded in an inappropriate manner. If the responses were a mix of appropriate and generalized responses, that individual was considered to have responded appropriately.

Two-tailed, 2x3 Fisher's exact tests (McDonald 2009) were used to compare responsiveness vs. unresponsiveness across all three playback treatments. Chi-squared goodness of fit tests (McDonald 2009) were then used as a follow up to look at responsiveness for each individual playback treatment. For those individuals that responded, two-tailed 2x3 Fisher's exact tests (McDonald 2009) were used to compare appropriate vs. generalized responses across all three playback treatments, and two-tailed 2x2 Fisher's exact tests (McDonald 2009) were used to compare appropriate vs. inappropriate responses between the thunder control and combined predator treatments (thunder control vs. eagle calls + ocelot growls), and between the two predator treatments (eagle calls vs. ocelot growls). A chi-squared goodness of fit test (McDonald 2009) was also used as a follow up to compare the proportion of appropriate vs. inappropriate responses to the predator treatments combined. The alpha level for all tests was set at 0.05.

RESULTS

Summary of Behaviors

Over the seven-week study, 24 playback trials were conducted on 16 different saki monkeys. Some individuals were tested on all three playback treatments, while others fled or appeared after the first or second playback. Thus, from these 16 individuals, eight eagle playback trials were tested on 12 individuals, nine ocelot playback trials were tested on 11 individuals, and seven thunder playback trials were tested on eight individuals (n=31). Behavioral responses for each playback treatment are summarized on **Table 2,** and **Figures 5** and **6**.

				Movement					Gaze			
Playback	No. of playbacks	No. of individuals	No response	Towards speaker	Away fror speaker	Up tree	Down tree	In denser cover	Towards speaker	Up	Down	Gazing around
Eagle	8	12	2	0	5	0	2	0	6	3	0	7
Ocelot	9	11	2	0	5	0	0	2	8	0	1	7
Thunder	7	8	0	0	4	1	2	0	7	4	4	7

Table 2: Overview of behavioral responses for each playback treatment. Responses are separated into directional movements and gaze. Row totals are not equal to the number of individuals because most sakis produced multiple behaviors for a single playback call.

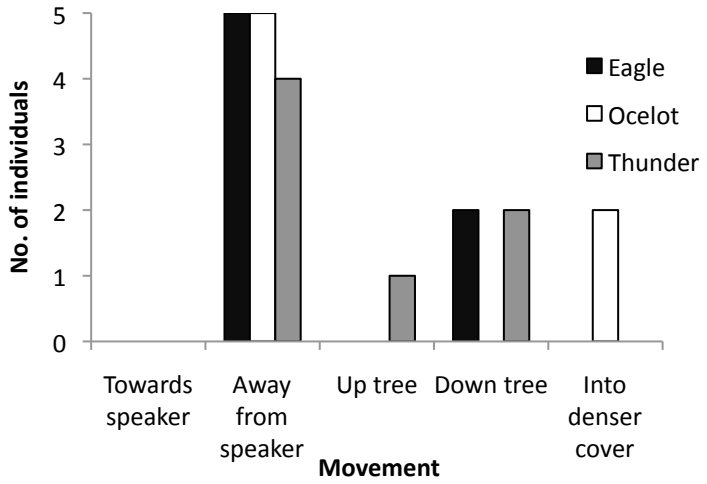

Figure 5: Horizontal and vertical movements given by sakis in response to the playback treatments. An individual had to move at least 2 consecutive steps in the same direction to classify a response as "movement".

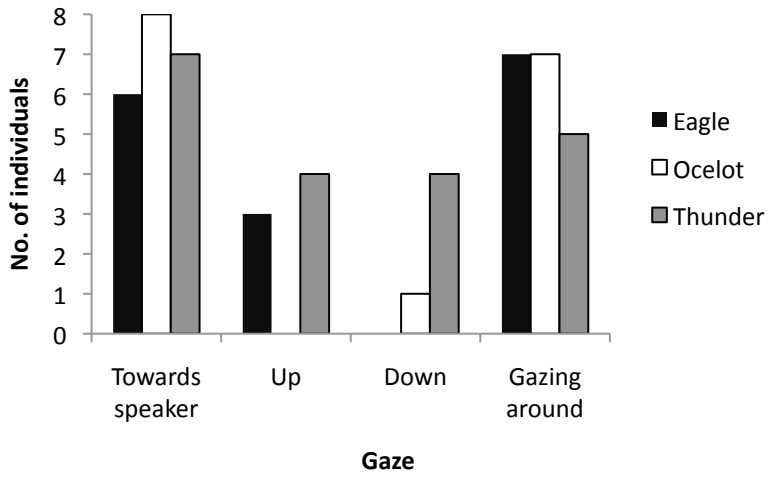

Figure 6: Directional gazes given by sakis in response to the playback treatments.

Responsiveness

There was no significant difference in the proportion of individuals that responded to the playbacks across all three playback treatments (2x3 Fisher's exact test, two-tailed, P=0.530), with individuals giving some type of 'movement' or 'gaze' response significantly more often than no response at all for each treatment (Eagle: 10 of 12 individuals responsive, x^2=5.333, p=0.021; Ocelot: 9 of 11 individuals responsive, x^2=4.455, p=0.035; Thunder control: 8 of 8 individuals responsive, x^2=8, p=0.005; **Table 3, Figure 7**). Because only eight individuals were measured for the thunder control, its expected value for the chi-squared goodness of fit test was four, thus violating the test's assumption that the expected value be at least five for at least 80% of the cells. Nevertheless, I decided to include the test result anyway due to the overall small sample size of the entire study and because no individual remained unresponsive to a thunder playback.

If an individual was responsive to a playback treatment, he or she typically responded with multiple behaviors by first scanning the area/looking around, then looking towards the speaker (i.e., the source of sound), and then finally producing a more

specific response (e.g., looking or moving in a specific direction). It was as if the monkey was first checking out the surrounding scene before deciding how to act most appropriately to the broadcasted sound.

Playback	No resp.	Any resp.
Eagle	2	10
Ocelot	2	9
Thunder	0	8

Table 3: Overview of responsiveness for each playback treatment. Individuals either acknowledged/responded to the playback with any of the 'movement' or 'gaze' behaviors listed in Table 2, or remained unresponsive.

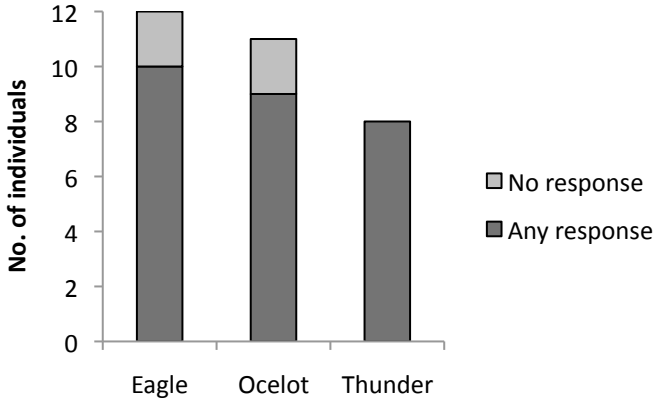

Figure 7: Comparison of responsiveness vs. unresponsiveness for each playback treatment.

Appropriate Responses

Appropriate and generalized responses occurred in equal proportions across all three playback treatments (2x3 Fisher's exact test, two-tailed, P=0.861, **Table 4, Figure 8**). However, when comparing appropriate to inappropriate responses, appropriate responses occurred significantly more often following the playbacks of predator calls compared to the playbacks of the thunder control (Eagle + Ocelot: 8 of 8 individuals responded appropriately; Thunder: 2 of 6 individuals responded appropriately; 2x2 Fisher's exact test, two-tailed, P=0.015; **Table 5, Figure 9**). This ultimately revealed thunder playbacks to be a poor control choice, and just seemed to confuse the monkeys more than actually reveal any relevant information.

While rate of appropriate vs. inappropriate responses differed between the predator and thunder control playbacks, no such difference was found when comparing the two predator playbacks to each other (Eagle: 5 of 5 individuals responded appropriately; Ocelot: 3 of 3 individuals responded appropriately; 2x2 Fisher's exact test, two-tailed, P=1.00; **Table 5, Figure 10**), with appropriate responses occurring significantly more than inappropriate responses for both predator playback treatments combined (Eagle + Ocelot: 8 of 8 individuals responded appropriately, x^2=8.00, p=0.005). In fact, no inappropriate response was ever given to either predator type (e.g., no individual ever gave a terrestrial predator response to an eagle call, or an aerial predator response to an ocelot growl). Thus while further confirmation is needed, the lack of inappropriate responses to either predator treatment suggests that saki monkeys may be capable of differentiating between aerial and terrestrial predators.

Playback	Approp. Resp.	General Resp.
Eagle	5	5
Ocelot	3	6
Thunder	2	2

Table 4: Overview of appropriate vs. generalized responses for each playback treatment. See Table 1 for definitions of appropriate and generalized responses.

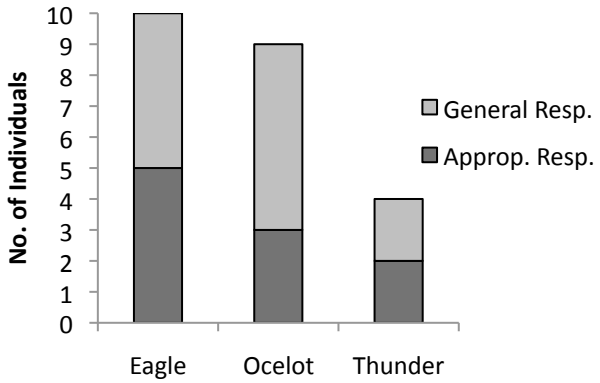

Figure 8: Comparison of appropriate vs. generalized responses for each playback treatment.

Playback	Approp. Resp.	Inapprop. Resp.
Eagle	5	0
Ocelot	3	0
Thunder	2	4

Table 5: Overview of appropriate vs. inappropriate responses for each playback treatment. See Table 1 for definitions of appropriate and inappropriate responses.

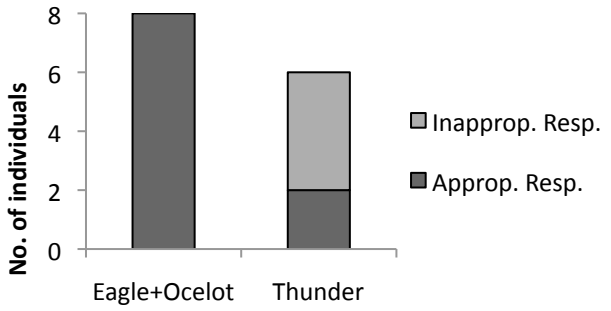

Figure 9: Comparison of appropriate vs. inappropriate responses between the predator and thunder control playback treatments.

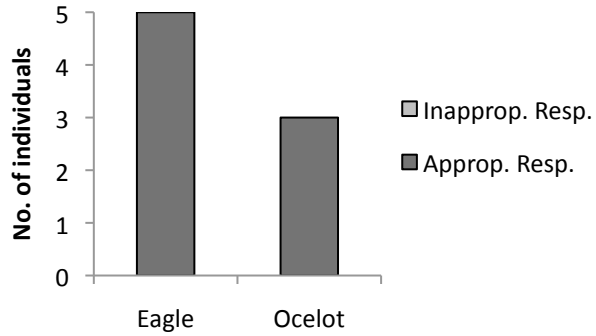

Figure 10: Comparison of appropriate vs. inappropriate responses between the two predator playback treatments. Note that no individual responded inappropriately to either predator call.

Vocalizations

There were only two instances out of the 24 playback trials where sakis vocally responded, thus the sample size of vocalizations was too small to analyze statistically. Of the two playback trials that did evoke vocal responses, one was for an ocelot growl and the other was for an eagle call. The ocelot growl caused multiple, hidden individuals to respond with a series of chits and chits with barks, while the eagle call caused two individuals to respond with a series of soft chits (vocalizations defined by Kieran 2012).

DISCUSSION

Summary

Recordings of harpy eagle calls (an aerial predator), ocelot growls (a terrestrial predator), and thunder claps (a control stimulus) were played to free-ranging groups of equatorial saki monkeys to determine how individuals vocally and behaviorally respond to predators, and whether they can respond differently to aerial and terrestrial predators. Response variables measured included individuals' vocalizations, movement, and gaze orientation. For both predator playback treatments (eagle calls and ocelot growls), we found that generalized-alerted or predator-specific responses were given more often than no response at all. More specifically, individuals typically responded with multiple behaviors by first scanning the area and looking towards the speaker (i.e., the source of sound), as if looking for further cues to a predator's presence (Seyfarth et al. 1980a), and then producing a more specific response (i.e., looking up or moving down for an eagle call, or looking down, moving up, or moving into denser cover for an ocelot growl). Although the sample size was small, individuals never responded with an incorrect anti-predator behavior for either predator treatment.

While predator calls seemed to evoke a more specific set of responses from the monkeys, the thunder control stimulus produced an array of behaviors from individuals (e.g., looking up, down and around). The sakis seemed more confused than anything else, often responding in ways that were inconsistent to what I defined as being "correct"/"appropriate" responses for this stimulus. For example, many would look down towards the ground upon hearing a thunder playback, but in a real life situation when it actually is thundering, it would make more sense for one to look up and scan the

sky rather than look down towards the ground. However, looking down towards the ground was essentially looking towards the direction from which the sound was coming from. Therefore, similar to what they seemed to do for the other two predator calls, they simply could have just been searching for additional cues to determine how to act most appropriately.

I realized after data collection that thunder was a poor choice for a control stimulus. The intention going into the study was to have thunder serve as a "startling sound" control treatment to match the two threatening predator treatments, in the hope that monkeys would remain unresponsive to thunder because it presents no actual danger to them. Overall, this was to ensure that the sakis were actually responding to the recordings of the predators, as opposed to just any sound coming from the speaker (Caselli et al. 2015). However, there were several times when the thunder control seemed to evoke more of an alarmed response than the actual predator calls themselves (e.g., immediate gaze and movement responses in every which direction). This could have been due to the loud and "sharp" features in the frequency and amplitude of the thunder sounds, which could directly influence the observed individuals' nervous systems and consequently impact their behavior (Morton 1977, Rendall et al. 2009). A more appropriate control would have been a non-threatening, background noise such as running water or morning sounds in a woodland forest.

Lack of Vocalizations

One behavioral aspect that was surprising was the sakis' lack of vocalizations/alarm calls following either predator playback treatment. Alarm calls are a common anti-predator strategy in primates as well as in many other vertebrates, and are

often used as a way to warn conspecifics of the nearby predator, and to signal to the predator itself that it been detected (Zuberbühler et al. 1997, Caro 2005, Cäsar and Zuberbühler 2012). Previous predator playback studies with primates have revealed that some species of primates produce different alarm calls for different predator types (e.g. Seyfarth et al. 1980a,b – vervet monkeys in Kenya; Zuberbühler et al. 1997 and Zuberbühler 2000 – Diana monkeys in Côte d'Ivoire; Fitchtel and Kappeler 2002 – redfronted lemurs (*Eulemur fulvus rufus*) and white sifakas (*Propithecus verreauxi verreauxi*) in Madagascar; Kirchhof and Hammerschmidt 2006 - saddleback and moustached tamarins (*Saguinus fuscicollis* and *Saguinus mystax*) in Peru; Schel et al. 2010 - guereza colobus monkeys in Uganda; Wheeler 2010 – tufted capuchin monkeys (*Cebus apella nigritus*) in Argentina; Cäsar et al. 2012 - black-fronted titi monkeys in Minas Gerais). All of these studies demonstrated the primates producing acoustically different alarm calls for their terrestrial and aerial predators, an important ability to have for warning conspecifics when escape modes are different for different predator classes (Kirchhof and Hammerschmidt 2006). Furthermore, de Luna et al.'s (2010) study examining predation attempts on red titi (*Callicebus discolor*) and equatorial saki monkeys in Ecuador found mobbing and loud alarm calling to be common anti-predator behaviors in saki groups. Because saki monkeys inhibit dense forests where visual communication is limited, vocal communication should play an important role in predator contexts (Cäsar et al. 2012).

My original plan for this study was to examine sakis' predator alarm calls more in depth to determine whether the calls contain referential meaning that other group members can understand. For alarm calls to have referential meaning, 1) each call must

have consistent structural acoustic differences that correspond with specific predators or predator classes, and 2) conspecifics must appropriately respond to a particular predator call just as they would if the predator were actually present (e.g., look up/move down for an eagle alarm call, look down/move up for an ocelot alarm call) (Zuberbühler et al. 1997). If group members responded in the same manner upon hearing a specific predator call or saki alarm call given to that predator, it would have ultimately revealed referential meaning in their alarm calls (Zuberbühler et al. 1997).

My proposed design for this study was to spend the first three weeks in the field playing predator calls to saki groups, with the presumption that individuals would respond with predator-specific alarm calls that I would be able to record and then play back to them in the last four weeks. However, due to the monkeys' predominately silent behavior whenever a predator call was played, I switched the methods so that I was only looking at whether sakis differentially reacted upon hearing a terrestrial vs. aerial predator within their territory.

One possible reason for the sakis' overall silent demeanor could have been because individuals were detecting and perceiving my field assistants and me as a threat. Zuberbühler et al. (1997) asserts that there are two types of predators: 1) "surprise" or "ambush" predators - those that rely on stealth and surprise attacks to capture prey (e.g., ocelots and eagles), and 2) "pursuit" predators - those that actively chase and pursue their prey until capture (e.g., human hunters). Producing loud alarm calls would be an effective way to inform an ambush-type predator that it has been detected, but a more effective strategy for a pursuit-type predator would be to remain cryptic in the canopy, so as to remain undetected (Zuberbühler et al. 1997). While regional hunting pressures have

declined for primates since the ACRCTT was established in 1991 (Newing and Bodmer 2003), humans may still be perceived as a threat if a species' adaptive anti-predator responses towards humans have not yet been removed by processes of natural selection (Papworth et al. 2013). Several other studies have found monkeys to remain cryptic or to silently flee upon encountering or hearing humans (e.g., Zuberbühler et al. 1997, Croes et al. 2006, Arnold et al. 2008, Papworth et al. 2013). Because we were essentially pursuing saki groups through the forest (at some points my guide, Manuel, would even chase them), the monkeys may have viewed us as pursuit-type hunters and thus focused on remaining silent and cryptic towards us, rather than respond to the sounds of other predator playbacks (i.e., predators they have not even visually detected yet).

While the sakis were, overall, vocally unresponsive to the playbacks, on two occasions individuals did respond vocally: 1) one ocelot playback trial caused several individuals to respond with a series of chits and chits with barks, and 2) one eagle playback trial caused two individuals to respond with a series of soft chits (vocalizations as defined by Kieran 2012). Furthermore, early on in this study, a tourist group at the TRARC reported an attempted black and white hawk eagle (*Spizaetus melanoleucus*) attack on a juvenile saki. The video recording they had of the saki group after the attack showed several individuals responding with a series of screams and barks (vocalizations as defined by Kieran 2012). Kieran's (2012) study on the vocalizations of equatorial saki monkeys in the ACRCTT found chits, barks, and screams to all occur in different encounter situations (e.g., encounters with humans, conspecifics, other primate species, or predators). Furthermore, Chism and Kieran (2014) found that equatorial sakis frequently vocalize with long sequences of several call types. Thus while a much more

thorough analysis is needed, it is possible that chits, barks, and screams, or a combination of them, could be used in different predator contexts.

Lack of Alarmed Movements

One other surprising behavioral aspect was the lack of alarmed movements given to many of the predator vocalizations. Prior to data collection, I assumed that saki monkeys would be much more eager to flee the area, or at least move higher or lower in the tree depending on the predator type. However, out of the 17 predator playbacks trials (eagle calls and ocelot growls), there were only seven instances where individuals moved away or in the appropriate up or down direction. For the ten other predator playback trials, individuals remained sitting, seemingly unalarmed, while only scanning the area and/or gazing in the appropriate direction. Thus, attempts at locating the predator seemed to be a much more common response than rapid escape. While increased vigilance and appropriate gaze orientation are considered anti-predator responses, these responses would not necessarily prevent an individual from being preyed on if a predator were actually present (e.g., failure to flee/escape if a predator were nearby would ultimately result in capture).

As discussed previously, the possibility of having cryptic behavior towards human presence could be one explanation for the lack of alarmed movements. However, these behaviors could also be explained by the threat-sensitivity hypothesis, which predicts that some prey posses the ability to assess predation risk and only respond to those situations that are perceived as being most threatening (Helfman 1989). Threat-sensitivity in animals has been demonstrated in several other studies (e.g., Robinson 1980, Helfman 1989, Chivers et al. 2001, Papworth et al. 2013, Etting and Isabel 2014).

Defensive and/or fleeing anti-predator behaviors have significant costs by expelling energy and taking away time needed for other important activities (e.g., feeding, grooming) (Lind and Cresswell 2005). Therefore, being able to distinguish threatening from non-threatening situations can have significant energy savings if an individual responds only to situations that pose as an actual threat (Papworth et al. 2013). In relating threat-sensitivity back to this study, the sakis' lack of alarmed movements to the predator playbacks may have been a result of some individuals being able to appropriately assess the situation and realize no predator nearby. Of course this is only a hypothesis, and an entirely separate study would have to be performed in order to determine whether the threat-sensitivity hypothesis applies to saki monkeys. Kirchhof and Hammerschmidt (2006) also suggest that fast gaze response into the appropriate direction, rather than rapid escape, could be a sufficient enough anti-predator strategy for determining whether escape is actually necessary (i.e., flexible anti-predator responses are more beneficial; it may not always be necessary to have a specific set of escape modes for different predators).

One final factor that could have influenced the lack of alarmed movements to predator playbacks was the rate of occurrence in which the playback trials took place. Cheney and Seyfarth (1990) and Fischer et al. (2013) assert that in order to get unbiased data, playback experiments must be as natural as possible, taking place at low rates so as to not reduce the response strength by habituating groups to recordings. Furthermore, Fischer et al. (2013) suggests that the most appropriate playback experiments should consist of no more than two trials on a single day (a test condition, and a control condition). For our study however, we attempted to present sakis with three playback

trials (both predator test conditions, and a control condition). We also presented each of these sounds shortly after one another. The mixture of different sound stimuli played immediately after one another may have just confused the sakis and reduced the overall strength in their responses. However, due to the difficulty in finding saki groups and limited amount of time we had in the field, conducting the playback experiments in this manner was our only option for acquiring an adequate sample size.

Conclusion

While there may have been some uncontrollable bias in the data collection process (e.g., poor control, too many sounds in a short amount of time, overexposure), sakis were nonetheless responsive to the predator playbacks and never responded with an inconsistent anti-predator behavior for either predator. Thus, my data provide preliminary evidence and support the hypothesis that equatorial sakis are able to recognize predators and discriminate between aerial and terrestrial predator classes.

REFERENCES

Altman J. 1974. Observational study of behavior: sampling methods. *Behaviour.* 49(3-4): 227-266.

Aquino R, Cornejo FM., Lozano EP, and Heymann EW. 2009. Geographic distribution and demography of *Pithecia aequatorialis* (Pitheciidae) in Peruvian Amazonia. *American Journal of Primatology.* 71: 964-968.

Arnold K, Pohlner Y, Zuberbühler K. 2008. A forest monkey's alarm call series to predator models. *Behavioral Ecology and Sociobiology*. 62: 549–559.

Bennett CL, Leonard S, and Carter S. 2001. Abundance, diversity, and patterns of distribution of primates on the Tapiche River in Amazonian Peru. *American Journal of Primatology.* 54: 119-126.

Berger J. 1998. Future prey: some consequences of the loss and restoration of large carnivores. In TM Caro (Ed.), *Behavioral ecology and conservation biology* (p. 80-100). New York: Oxford University Press.

Berger J, Swenson JE, Persson IL. 2001. Recolonizing carnivores and naive prey: conservation lessons from Pleistocene extinctions. *Science.* 291: 1036–1039.

Birkhead TR, and P Monaghan. 2010. Ingenious ideas: the history of behavioral ecology. In DF Westneat, and CW Fox (Eds.), *Evolutionary behavioral ecology* (p. 3-16). Oxford: Oxford University Press.

Blumstein DT, Cooley L, Winternitz J, and Daniel JC. 2008. Do yellow-bellied marmots respond to predator vocalizations? *Behavioral Ecology and Sociobiology*. 62: 457-468.

Blumstein DT, Daniel JC, Griffin AS, Evans CS. 2000. Insular tammar wallabies (*Macropus eugenii*) respond to visual but not acoustic cues from predators. *Behavioral Ecology*. 11: 528-535.

Brush JA, and MA Norconk. 1999. Early behavioral development in a wild white-faced saki monkey (*Pithecia pithecia*). *American Journal of Physical Anthropology.* 28 (Suppl.): 99.

Buzzell CA, and JA Brush. 2000. Ontogeny of independence in wild and captive white-faced sakis (*Pithecia pithecia*). *American Journal of Primatology*. 51(Suppl. 1): 49-50.

Cantwell LR. 2010. Response of *Anolis sagrei* to acoustic calls from predatory and non-predatory birds. Proceedings of the National Conference on Undergraduate Research. University of North Carolina at Asheville.

Caro T. 2005. *Antipredator defenses in birds and mammals*. Chicago: The University of Chicago Press.

Cäsar C, and K Zuberbühler. 2012. Referential alarm calling behaviour in New World primates. *Current Zoology.* 58(5): 680-697.

Cäsar C, Byrne RW, Hoppitt W, Young RJ, and Zuberbühler K. 2012. Evidence for semantic communication in titi monkey alarm calls. *Animal Behaviour.* 84: 405-411.

Caselli CB, Mennill DJ, Gestich CC, Setz EF, and Bicca-Marques JC. 2015. Playback responses of socially monogamous black-fronted titi monkeys to simulated solitary and paired intruders. *American Journal of Primatology.* 77: 1135-1142.

Cheney DL, and RM Seyfarth. 1982. How vervet monkeys perceive their grunts: field playback experiments. *Animal Behavior.* 30: 739-751.

Cheney DL, and RM Seyfarth. 1988. Assessment of meaning and the detection of unreliable signals by vervet monkeys. *Animal Behavior.* 36: 477-486.

Cheney DL, and RM Seyfarth. 1990. *How monkeys see the world: inside the mind of another species.* Chicago: The University of Chicago Press.

Chism J, and TJ Kieran. 2014. Vocal communication of equatorial sakis (*Pithecia aequatorialis*) in Northeastern Peru. Presentation, XXIV Congress of the International Primatological Society, Hanoi, Vietnam.

Chism J, Brasington L, Kieran TJ, Santillan AD. In review. Sympatry of *Pithecia* species south of the Amazon River in Peru.

Chivers DP, Mirza RS, Bryer PJ, and Kiesecker JM. 2001. Threat-sensitive predator avoidance by slimy sculpins: understanding the importance of visual versus chemical information. *Canadian Journal of Zoology.* 79: 867-873.

Croes BM, Laurance WF, Lahm SA, Tchignoumba L, Alonso A, Lee ME, Campbell P, Buij R. 2006. The influence of hunting on antipredator behavior in central African monkeys and duikers. *Biotropica*. 39(2): 257–263.

de Luna AG, Sanmiguel R, and Di Fiore A. 2010. Predation and predation attempts on red titi monkeys (*Callicebus discolor*) and equatorial sakis (*Pithecia aequatoralis*) in Amazonian Ecuador. *Folia Primatologica*. 81: 86-95.

Di Fiore A, Fernandez-Duque E, and Hurst D. 2007. Adult male replacement in socially monogamous equatorial saki monkeys (*Pithecia aequatoralis*). *Folia Primatologica.* 78: 88-98.

Etting SF, and LA Isbell. 2014. Rhesus macaques (*Macaca mulatta*) use posture to assess level of threat from snakes. *Ethology.* 120: 1177-1184.

Fericean ML, Rada O, and Badilita M. 2015. The history and development of ethology. *Research Journal of Agricultural Science.* 47(2): 45-51.

Fernandez-Duque E, Di Fiore A, and de Luna AG. 2013. Pair-mate relationships and parenting in equatorial saki monkeys (*Pithecia aequatorialis*) and red titi monkeys (*Callicebus discolor*) of Ecuador. In LM Veiga, AA Barnett, SF Ferrari, and MA Norconk (Eds.), *Evolutionary biology and conservation of titis, sakis and uacaris* (p. 295-302). New York: Cambridge University Press.

Ferrari SF, Veiga LM, Pinto LP, Marsh LK, Mittermeier RA, and Rylands AB. 2013. Family Pitheciidae (titis, sakis, and uakaris). 2013. In RA Mittermeier, AB Rylands, and DE Wilson (Eds.), *Handbook of the mammals of the world* (Vol. 3: Primates) (p. 432-477). Barcelona: Lynx Edicions.

Fichtel C, and PM Kappeler. 2002: Anti-predator behavior of group-living Malagasy primates: mixed evidence for a referential alarm call system. *Behavioral Ecology and Sociobiology*. 51: 262-275.

Fischer J, Noser R, and Hammerschmidt K. 2013. Bioacoustic field research: a primer to acoustic analyses and playback experiments with primates. *American Journal of Primatology.* 75: 643-663.

Ghalambor CK, Angeloni LM, and Caroll SP. 2010. Behavior as phenotypic plasticity. In DF Westneat, and CW Fox (Eds.), *Evolutionary behavioral ecology* (p. 90-107). Oxford: Oxford University Press.

Griffin AS, Blumstein DT, Evans CS. 2000. Training captive-bred or translocated animals to avoid predators. *Conservation Biology.* 14: 1317-1326.

Griffin AS, Evans CS, Blumstein DT. 2001. Learning specificity in acquired predator recognition. *Animal Behavior*. 62: 577-589.

Hakansson G, and J Westander. 2013. *Communication in humans and other animals* (p. 1-46). Amsterdam: John Benjamins Publishing Company.

Happel RE. 1982. Ecology of *Pithecia hirsuta* in Peru. *Journal of Human Evolution.* 11: 581-590.

Helfman GS. 1989. Threat-sensitive predator avoidance in damselfish–trumpetfish interactions. *Behavioral Ecology and Sociobiology*. 24: 47–58.

Hershkovitz P. 1979. The species of sakis, genus *Pithecia* (Cebidae, Primates) with notes on sexual dichromatism. *Folia Primatologica*. 31:1–22.

Hershkovitz P. 1987. The taxonomy of South American sakis, genus *Pithecia* (Cebidae, Platyrrhini): a preliminary report and critical review with the description of a new species and a new subspecies. *American Journal of Primatology*. 12:387–468.

Hettena, AM, Munoz N, and Blumstein DT. 2014. Prey responses to predator's sounds: a review and empirical study. *Ethology.* 120: 1-26.

Heymann EW, Encarnacion F, and Canaquin JE. 2002. Primates of the Rio Curaray, Northern Peruvian Amazon. *International Journal of Primatology.* 23(1): 191-201.

Jones ME, Smith GC, Jones SM. 2004. Is anti-predator behaviour in Tasmanian eastern quolls (*Dasyurus viverrinus*) effective against introduced predators? *Animal Conservation*. 7:155–160

Kay RF, Meldrum DJ, and Takai M. 2013. Pitheciidae and other platyrrhine seed predators. In LM Veiga, AA Barnett, SF Ferrari, and MA Norconk (Eds.), *Evolutionary biology and conservation of titis, sakis and uacaris* (p. 3-12). New York: Cambridge University Press.

Kieran T. 2012. Identity and vocal repertoire of a Peruvian saki monkey (*Pithecia aequatorialis*). Masters Thesis. Winthrop University.

Kirchhof J, and K Hammerschmidt. 2006. Functionally referential alarm calls in tamarins (*Saguinus fusciocollis* and *Saguinus mystax*): evidence from playback experiments. *Ethology*. 112: 346-354.

Koda H, and H Sugiura. 2010. The ecological design of the affiliative vocal communication style in wild Japanese macaques: behavioral adjustments to social contexts and environments. In N Nakagawa, M Iakarnichi, and H Sugiura (Eds.), *The Japanese macaques* (p. 167-190). Tokyo: Springer.

Kvist P, and G Nebel. 2001. A review of Peruvian flood plain forests: ecosystems, inhabitants and resource use. *Forest Ecology and Management.* 150: 3-26.

Lehtonen E. 2016. The behavioural ecology of a potentially undescribed morph of saki monkey (genus *Pithecia*) in a highly diverse primate community. Masters Thesis. Uppsala University.

Lind J, and W Cresswell. 2005. Determining the fitness consequences of antipredation behavior. *Behavioral Ecology*. 16: 945–956.

Lohrey AK, Clark DL, Gordon SD, and Uetz GW. 2009: Antipredator responses of wolf spiders (Araneae: Lycosidae) to sensory cues representing an avian predator. *Animal Behavior*. 77: 813-821.

Lorenz K. 1981. *The foundations of ethology.* New York: Springer-Verlag.

Maciej P, Patzelt A, Ndao I, Hammerschmidt K, and Fischer J. 2013. Social monitoring in a multilevel society: a playback study with male Guinea baboons. *Behavioral Ecology and Sociobiology*. 67: 61-68.

Marsh LK. 2014. A taxonomic revision of the saki Monkeys, *Pithecia* Desmarest,1804. *Neotropical Primates.* 21(1): 1-165.

Marsh LK, and LM Veiga. 2008. *Pithecia aequatoralis.* The IUCN Red List of Threatened Species 2008: e.T17402A7013094. Retrieved from http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T17402A7013094.en.

McDonald JH. 2009. *Handbook of Biological Statistics* (2nd ed.). Baltimore: Sparky House Publishing.

Morton, E.S. 1977. On the occurrence and significance of motivation-structural rules in some bird and mammal sounds. *The American Naturalist.* 111(981): 855-869.

Newing H. 2009. Unpicking 'community' in community conservation: implications of changing settlement patterns and individual mobility for the Tamshiyacu Tahuayo Communal Reserve, Peru. In MN Alexiades (Ed.), *Mobility and migration in Indigenous Amazonia: contemporary ethnoecological perspectives* (p. 97-114). New York: Berghahn Books.

Newing H, and R Bodmer. 2003. Collaborative wildlife management and adaptation to change: the Tamshiyacu Tahuayo Communal Reserve, Peru. *Nomadic Peoples.* 7(1): 110–122.

Norconk MA. 2006. Long-term study of group dynamics and female reproduction in Venezuelan *Pithecia pithecia. International Journal of Primatology.* 27(3): 653-674.

Norconk MA. 2007. Sakis, uakaris, and titi monkeys. In CJ Campbell, A Fuentes, K MacKinnon, M Panger, and S Bearder (Eds.), *Primates in Perspective* (p. 123-138). New York: Oxford University Press.

Oliveira JMS, Lima MG, Bonvincino C, Ayres JM, and Fleagle JG. 1985. Preliminary notes on the ecology and behavior of the Guianan saki (*Pithecia pithecia*, Linnaeus 1766; Cebidae, Primate). *Acta Amazonica*. 15(1-2): 249–263.

Papworth S, Milner-Gulland EJ, Slocombe K. 2013. Hunted woolly monkeys (*Lagothrix poeppigii*) show threat-sensitive responses to human presence. *PLOSone*. 8(4): e62000.

Penn J. 2009. 2009 RCF Update. Blog. *Rainforest Conservation Fund.* Retrieved from http://www.rainforestconservation.org/hello-world/.

Peres CA. 1993. Notes on the ecology of buffy saki monkeys (*Pithecia albicans*, Gray 1860): a canopy seed-predator. *American Journal of Primatology.* 31: 129-140.

Pinto LP, Barnett AA, Bezerra BM, Boubli JP, Bowler M, de Alcântara Cardoso N, Caselli CB, Rodríguez MJO, Santos RR, Setz EZF, and Veiga LM. 2013. Why we know so little: the challenges of fieldwork on the Pitheciids. In LM Veiga, AA Barnett, SF Ferrari, MA Norconk (Eds.), *Evolutionary biology and conservation of titis, sakis and uacaris* (p.145-150). New York: Cambridge University Press.

Porter AM, Grote MN, Isbell LA, Fernandez-Duque E, Di Fiore A. 2015. A saki saga: dynamic and disruptive relationships among *Pithecia aequatorialis* in Ecuador. *Folia Primatologica.* 86: 455-473.

Prance GT. 1979. Notes on the vegetation of Amazonia III. The terminology of Amazonian forest types subject to inundation. *Brittonia*. 31(1): 26-38.

Puertas P, and RE Bodmer. 1993. Conservation of high diversity primate assemblage. *Biodiversity and Conservation.* 2: 586-593.

Rendall D, Owren MJ, and Ryan MJ. 2009. What do animal signals mean? *Animal Behaviour.* 78: 233-240.

Robinson SR. 1980. Antipredator behaviour and predator recognition in belding's ground squirrels. *Animal Behavior.* 28: 840-852.

Schel AM, and K Zuberbühler. 2009. Responses to leopards are independent of experience in Guereza colobus monkeys. *Behaviour.* 146:1709-1737.

Schel AM, Candiotti A, and Zuberbühler K. 2010. Predator-deterring alarm call sequences in Guereza colobus monkeys are meaningful to conspecifics. *Animal Behaviour.* 80: 799-808.

Seyfarth RM, and DL Cheney. 1990. The assessment by vervet monkeys of their own and another species' alarm calls. *Animal Behavior.* 40: 754-764.

Seyfarth RM, Cheney DL, and Marler P. 1980a. Vervet monkey alarm calls: semantic communication in a free-ranging primate. *Animal Behaviour*. 28:1070-1094.

Seythrth RM, Cheney DL, and Marler P. 1980b. Monkey responses to three different alarm calls: evidence for predator classification and semantic communication. *Science*. 210: 801-803.

Shettleworth SJ. 2010. *Cognition, evolution, and behavior.* New York: Oxford University Press.

Thompson CL. 2011. Sex, aggression, and affiliation: the social system of white-faced saki monkeys (*Pithecia pithecia*). Ph.D. Dissertation. Kent State University.

Thompson CL. 2013. Non-monogamous copulations and potential within-group mating competition in white-faced saki monkeys (*Pithecia pithecia*). *American Journal of Primatology.* 78: 817-824.

Thompson CL. 2016. To pair or not to pair: sources of social variability with white-faced saki monkeys (*Pithecia pithecia*) as a case study. *American Journal of Primatology.* 78: 561- 572.

Thompson CL, Norconk MA, and Whitten PL. 2012. Why fight? Selective forces favoring between-group aggression in a variably pair-living primate, the white-faced saki (*Pithecia pithecia*). *Behaviour.* 149: 795-820.

Thuppil V, and RG Coss. 2013. Wild Asian elephants distinguish aggressive tiger and leopard growls according to perceived danger. *Biology Letters*. 9, 20130518.

Tinbergen N. 1951. *The study of instinct.* New York: Oxford University Press.

Van Belle S, Fernandez-Duque E, Di Fiore A. 2016. Demography and life history of wild red titi monkeys (*Callicebus discolor*) and equatorial sakis (*Pithecia aequatorialis*) in Amazonian Ecuador: a 12-year study. *American Journal of Primatology.* 78: 204-215.

Vié, JC, Richard-Hansen C, and Fournier-Chambrillon C. 2001. Abundance, use of space, and activity patterns of white-faced sakis (*Pithecia pithecia*) in French Guiana. *American Journal of Primatology*. 55: 203–221.

von Frisch K. 1954. *The Dancing Bees: an account of the life and senses of the honey bee.* London: Methuen.

Wheeler BC. 2010. Production and perception of situationally variable alarm calls in wild tufted capuchin monkeys (*Cebus apella nigritus*). *Behavioural Ecology and Sociobiology.* 64: 989-1000.

Wiley RH. 2003. Is there an ideal behavioural experiment? *Animal Behaviour.* 66: 585-588.

Ydenberg C, and LM Dill. 1986. The economics of fleeing from predators. In JS Rosenblatt, C Beer, MC Busnell, and PJB Slater (Eds.), *Advances in the Study of Behavior* (Vol 16.) (p. 229-249). New York: Academic Press Inc.

Zuberbühler K. 2000. Causal knowledge of predators' behaviour in wild Diana monkeys. *Animal Behaviour*. 59: 209-220.

Zuberbühler K, Noë R, and Seyfarth RM. 1997. Diana monkey long-distance calls: messages for conspecifics and predators. *Animal Behaviour.* 53: 585-604.